

CLAIMS

1. An isolated polynucleotide encoding a multifunctional germacrene-D synthase.
- 5 2. An isolated polynucleotide having the sequence of SEQ ID NO:1 or a fragment or variant thereof encoding a polypeptide with multifunctional germacrene-D synthase activity.
3. A polynucleotide as claimed in claim 1 or claim 2 wherein the polynucleotide is
10 capable of facilitating the conversion of FDP to a mixture of germacrene-D and one or more other sesquiterpenes selected from *delta*-cadinene, *delta*-elemene, elemol, *gamma*-muurolene, *gamma*-cadinene, *gamma*-elemene and germacrene B.
4. An isolated polynucleotide as claimed in claim 3 wherein the sequence has at
15 least 60% identity to the nucleotide sequence of SEQ ID NO:1.
5. An isolated polynucleotide as claimed in claim 3 wherein the sequence has at least 90% identity to the nucleotide sequence of SEQ ID NO:1.
- 20 6. An isolated polynucleotide as claimed in claim 3 wherein the sequence has at least 95% identity to the nucleotide sequence of SEQ ID NO:1.
7. An isolated polynucleotide as claimed in claim 3 wherein the nucleotide sequence is that of SEQ ID NO:1.
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8. An isolated polynucleotide encoding the polypeptide of SEQ ID NO:2 or encoding a variant or a fragment of that sequence which has a multifunctional germacrene-D synthase activity.
- 30 9. An isolated polynucleotide as claimed in claim 8 wherein the polypeptide has at least 60% identity with the amino acid sequence of SEQ ID NO:2.

10. An isolated polynucleotide as claimed in claim 8 wherein the polypeptide has at least 90% identity with the amino acid sequence of SEQ ID NO:2.
11. An isolated polynucleotide as claimed in claim 8 wherein the polypeptide has at least 95% identity with the amino acid sequence of SEQ ID NO:2.
12. An isolated polynucleotide as claimed in claim 8 wherein the polypeptide has the sequence of SEQ ID NO:2.
13. An isolated multifunctional germacrene-D synthase polypeptide.
14. An isolated multifunctional germacrene-D synthase having the sequence of SEQ ID NO:2 or a fragment or variant thereof with multifunctional germacrene-D synthase activity.
15. An isolated multifunctional germacrene-D synthase as claimed in claim 14 wherein the amino acid sequence has at least 60% identity with the sequence of SEQ ID NO:2.
16. An isolated multifunctional germacrene-D synthase as claimed in claim 14 wherein the amino acid sequence has at least 90% identity with the sequence of SEQ ID NO:2.
17. An isolated multifunctional germacrene-D synthase as claimed in claim 14 wherein the amino acid sequence has at least 95% identity with the sequence of SEQ ID NO:2.
18. An isolated multifunctional germacrene-D synthase as claimed in claim 14 wherein the amino acid sequence is a mature sequence derived from SEQ ID NO:2.
19. A genetic construct comprising a polynucleotide of any one of claims 1 to 12.

20. A genetic construct comprising an open reading frame polynucleotide encoding a polypeptide of any one of claims 13 to 18.
21. A genetic construct as claimed in claim 20 further comprising a promoter
5 sequence.
22. A genetic construct as claimed in claim 21 which further comprises a termination sequence.
- 10 23. A genetic construct as claimed in claim 22 wherein the sequence of the encoded polypeptide has the amino acid sequence of SEQ ID NO:2 or a fragment thereof with multifunctional germacrene-D activity.
24. A genetic construct comprising in the 5'-3' direction a polynucleotide which
15 hybridizes to a polynucleotide encoding a polypeptide of any one of claims 13 to 18.
25. A genetic construct as claimed in claim 24 further comprising a promoter sequence.
- 20 26. A genetic construct as claimed in claim 25 which comprises a termination sequence
27. A genetic construct as claimed in claim 26 wherein the sequence of the encoded polypeptide has the amino acid sequence of SEQ ID NO:2 or a fragment thereof with
25 multifunctional germacrene-D activity.
28. A vector comprising a genetic construct of any one of claims 19 to 27.
29. A host cell comprising a genetic construct of any one of claims 19 to 27.
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30. A transgenic plant cell which includes a genetic construct of any one of claims 19 to 27.

31. A transgenic plant comprising a plant cell as claimed in claim 30.
32. A method of preparing germacrene-D, *delta*-cadinene, *gamma*-cadinene, *gamma*-muurolene, *gamma*-elemene, *delta*-elemene, elemol or germacrene B comprising the steps of
- 5 (a) culturing a cell which has been genetically modified with a polynucleotide any one of claims 1-12 to provide increased multifunctional germacrene-D synthase activity;
- (b) providing the cell with farnesyl diphosphate or geranyl diphosphate if
- 10 necessary; and
- (c) separating the germacrene-D and/or *delta*-cadinene and/or *delta* elemene and/or elemol and/or germacrene B, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene produced.
- 15 33. A method for modulating the Germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene production of a plant, the method comprising: increasing or decreasing expression of multifunctional germacrene-D synthase wherein said increasing or decreasing is achieved by genetic modification to
- 20 alter the expression of a gene encoding a multifunctional germacrene-D synthase.
34. A method as claimed in claim 33 wherein the synthase comprises a synthase with the sequence of SEQ ID NO: 2.
- 25 35. A method for modulating germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene production in a plant, the method comprising of :
- (a) introducing into the plant, a genetic construct of claims 19-27; and
- 30 (b) transcriptionally expressing the polynucleotide in the plant.

36. A method for modulating germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene production in a plant, the method comprising of
- 5 (a) introducing into the plant, a DNA genetic construct of claims 19-27; and
(b) expressing the polypeptide in the plant.
37. A polynucleotide having at least 15 contiguous nucleotides from SEQ ID NO: 1
- 10 38. A method of selecting a plant with altered germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or *gamma*-muurolene, and/or *gamma*-elemene content comprising the steps of:
(a) contacting polynucleotides from at least one plant with at least one polynucleotide comprising at least 15 contiguous nucleotides of the polynucleotide of
15 claim 1 to assess the expression of multifunctional germacrene-D synthase; and
(b) selecting a plant showing altered expression.
39. A method as claimed in claim 38 wherein the polynucleotide has at least 15 contiguous nucleotides from a sequence selected from SEQ ID NO: 1, SEQ ID NO: 3,
20 SEQ ID NO: 4 and SEQ ID NO: 7 and the plant is a plant of the genus *Actinidia*.
40. A method as claimed in claim 38 wherein the plant is a plant of the genus *Vaccinium*.
- 25 41. A method for preparing a sesquiterpene comprising:
(a) obtaining a polypeptide as claimed in any one of claims 13-18; and
(b) incubating farnesyl diphosphate in the presence of the polypeptide, and
(c) separating the germacrene-D and/or *delta*-cadinene and/or germacrene B and or elemol and/or *delta*-elemene, and/or *gamma*-cadinene, and/or
30 *gamma*-muurolene, and/or *gamma*-elemene produced.

42. A method as claimed in claim 41 wherein the products of step (b) are trapped in a matrix providing an acid environment.

43. A method as claimed in claim 42 wherein the matrix is a silica base matrix.

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44. A method as claimed in claim 41 wherein the sesquiterpene is germacrene D.